The Exposome and Adductomics: Promises and Pitfalls

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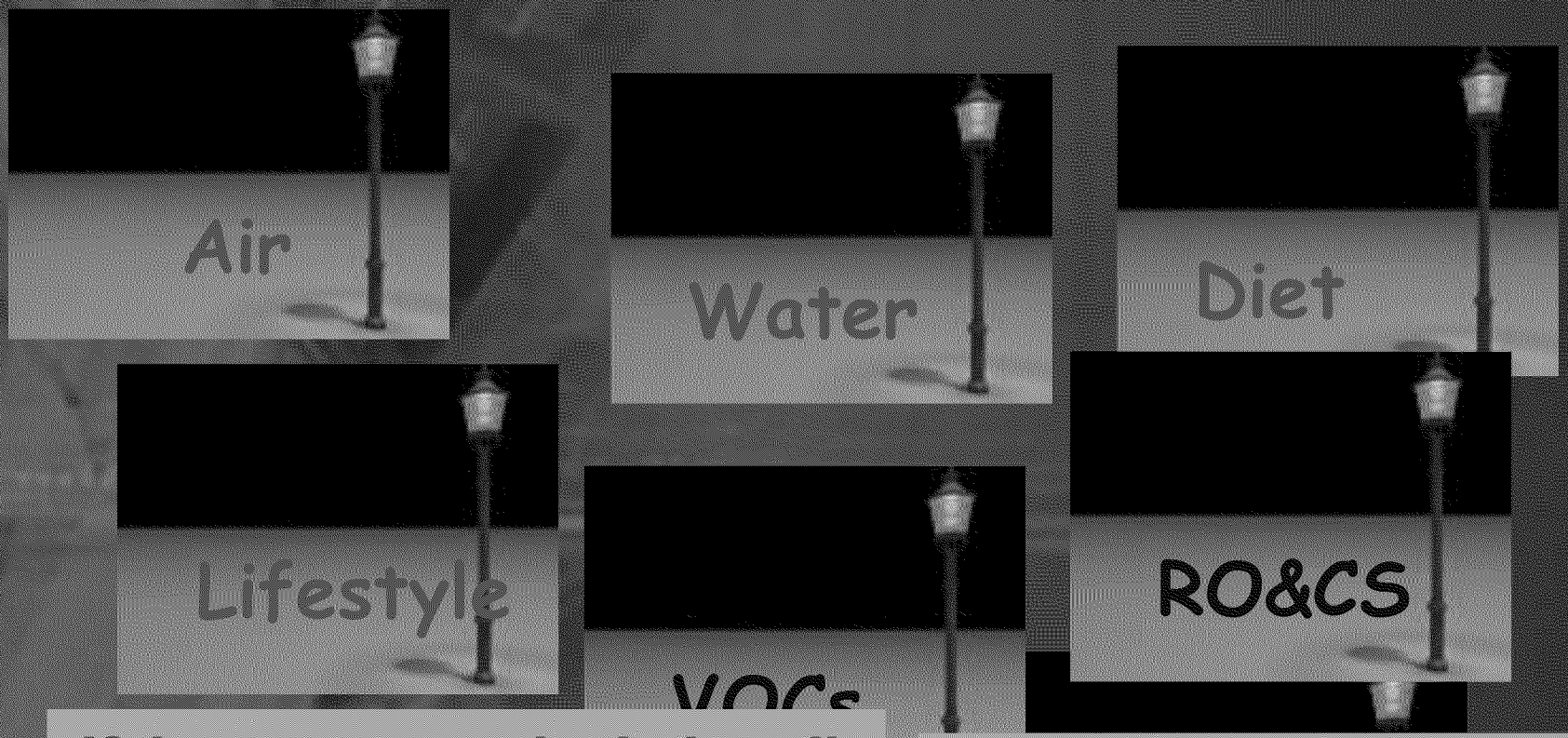
The exposome shifts the paradigm



If the exposome includes all chemicals - from all sources,

then how should we characterize exposures for health studies?

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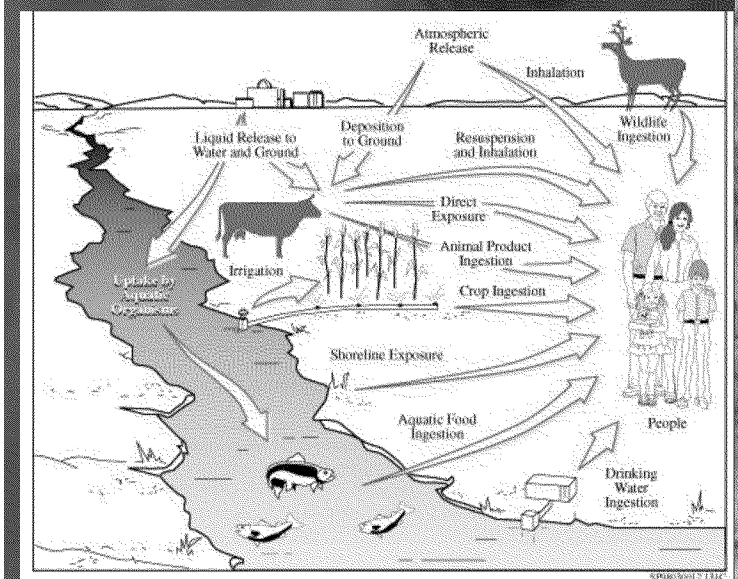
Bottom-up exposomics

- Large sampling effort
- Ignores endogenous sources of exposure



Courtesy United States Department of Energy, Hanford site

Evaluate uptake, metabolism, etc. of *important* agents (to estimate dose)

Test for associations with case status

Measure all analytes in air, water, food, etc. from cases and controls

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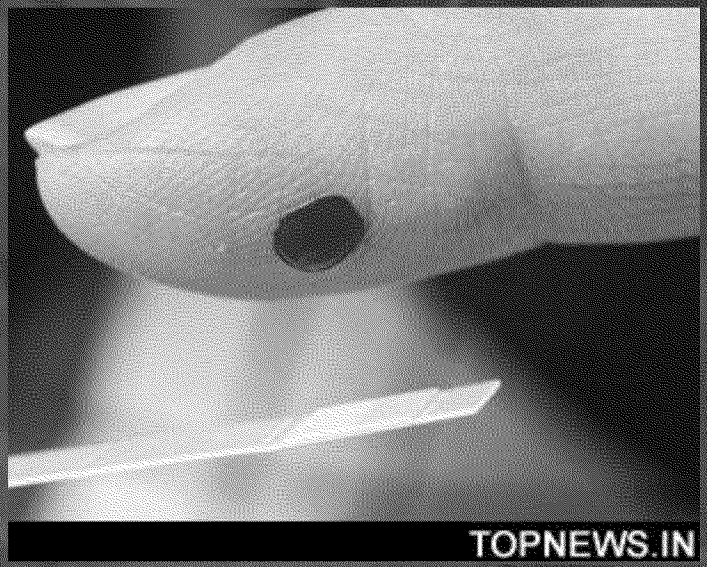
Top-down exposomics



- Relevant to internal dose
- Manageable sampling effort
- Includes all sources

Measure all analytes in blood from cases and controls

⬇

Test for associations with case status

⬇

Identify *important* agents and determine sources of exposure

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Reducing the universe of chemicals

"The studies in recent years … indicate to us that the formation … of alkylating or arylating structures in chemical carcinogenesis is emerging as a central, but not necessarily universal, theme".

EC Miller and JA Miller, 1966, *Pharmacol Rev* 18:806

"The liver … can convert stable organic compounds to potent alkylating agents … that … cause damage to liver, kidney, bone marrow, and other tissues… Similarly, allergic responses … may be mediated by the reaction of body proteins with trace amounts of chemically active … metabolites".

BB Brodie *et al*., 1971, *PNAS* 68:160

Alkylating agents are *electrophiles* (*'electron lovers'*) that react with *nucleophiles* (electron-rich substances), including DNA and proteins. The Millers and Brodie were among the fist to recognize that toxicants are generally electrophiles.

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Focus upon electrophiles

"Although there are important exceptions, the vast majority of effects attributed to reactive toxicity arise from the reaction between an electrophilic chemical and a biological nucleophile."

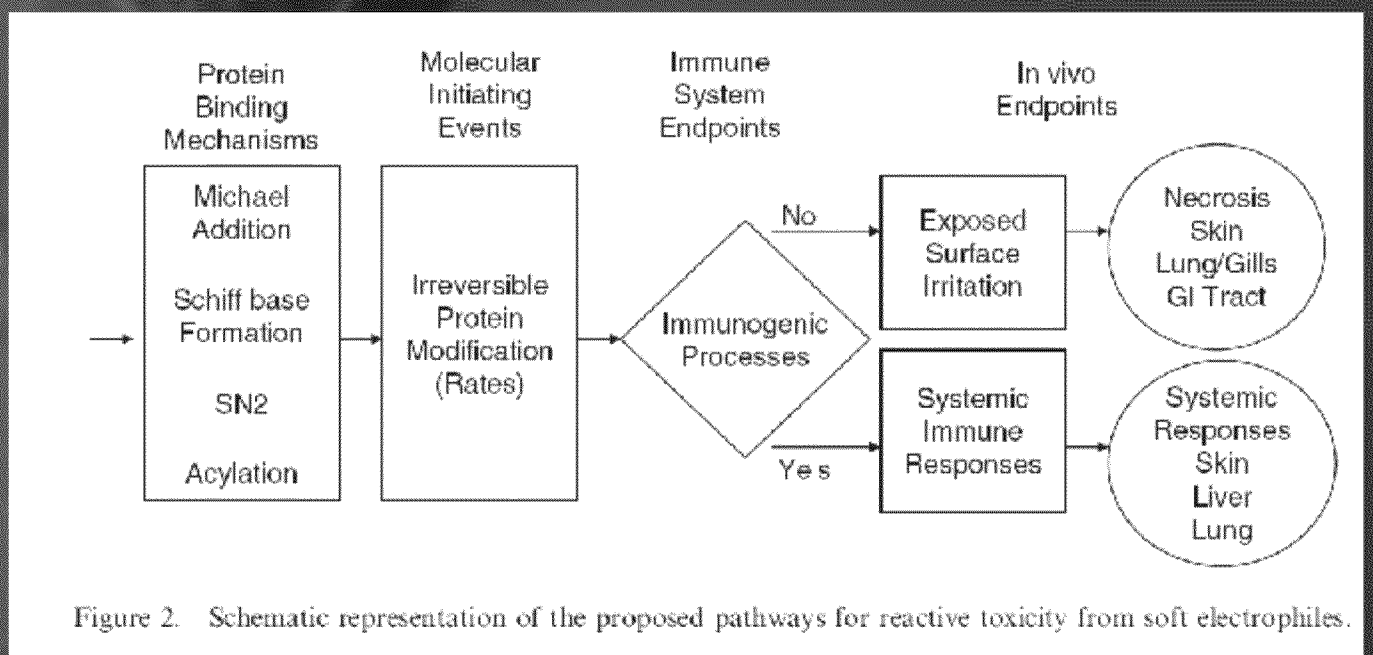

Figure 2. Schematic representation of the proposed pathways for reactive toxicity from soft electrophiles.

Schultz, TW, *et al., 2006, SAR and QSAR Environ Res*, 17(4): 413-428.

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Adductomics: characterizing exposures to electrophiles

- Electrophiles have short life spans in vivo
  - E.g., alkylating agents, aldehydes, epoxides, quinones, reactive oxygen species, reactive carbonyl species
- But they form stable adducts (addition products) by reacting with biological nucleophiles (e.g., DNA, proteins, glutathione)
- By measuring all adducts of a given nucleophile we perform 'adductomics'
  - Triple quadrupole MS with selected reaction monitoring (SRM) has the necessary sensitivity and specificity

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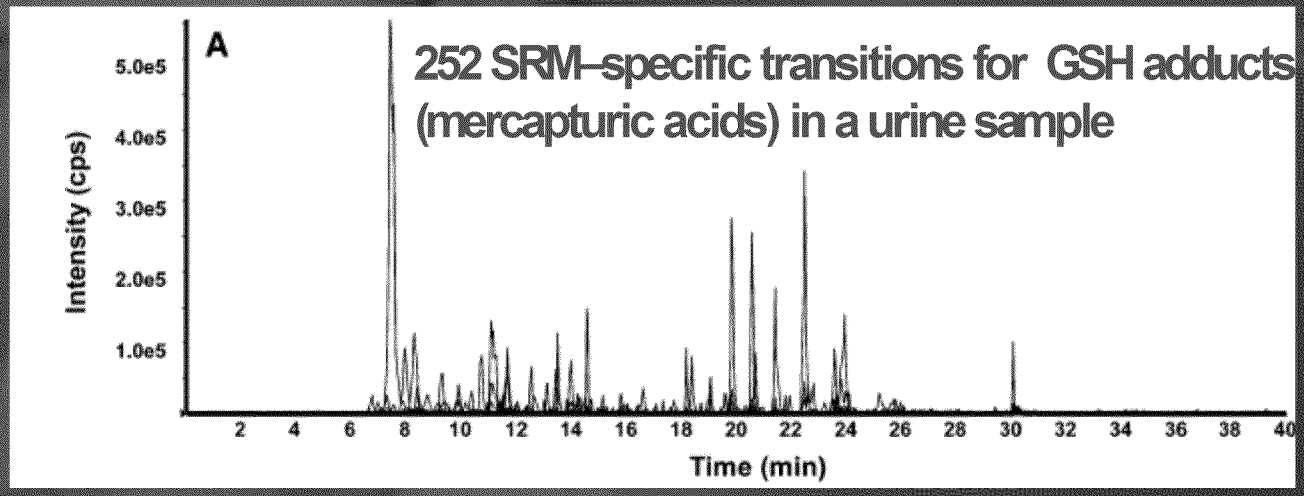
Glutathione adductomics: an extension of metabolomics

Anal. Chem. **2007**, *79*, 2918−2926

Tools in Metabonomics: An Integrated Validation Approach for LC-MS Metabolic Profiling of Mercapturic Acids in Human Urine

Silvia Wagner,† Karoline Scholz,† Maximilian Sieber,† Marco Kellert,† and Wolfgang Voelkel†,‡,*



GSH adducts are transient (vary greatly within days)

DNA adductomics

ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 5 & 6, 2006
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Requires 100 μg DNA (equivalent to 20 ml blood)

Forum Original Research Communication

Development of the Adductome Approach to Detect DNA Damage in Humans

ROBERT A. KAN
HIROKAZU T

374 SRM-specific transitions for DNA adducts in human lung tissue

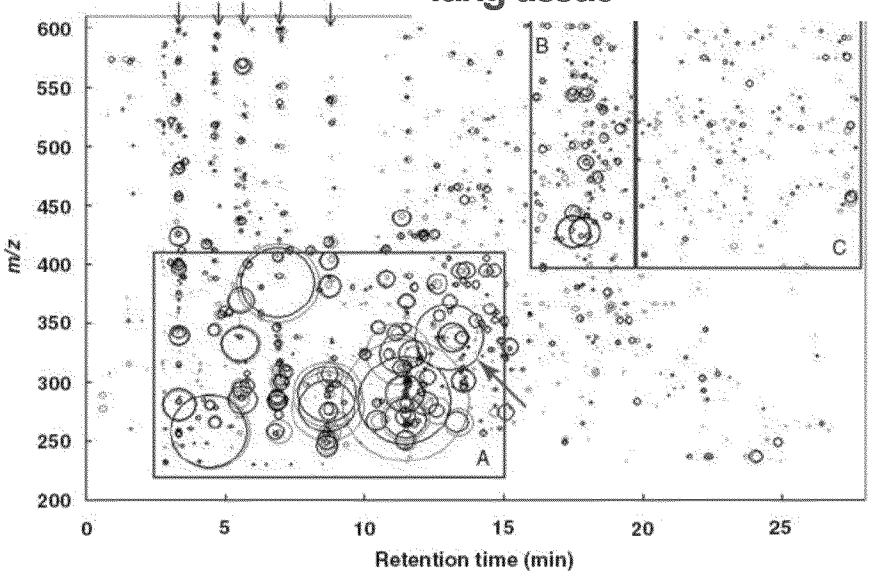

FIG. 2. Adductome map of putative DNA adducts detected in human lung tissue from a nonsmoker (*light circles*) and a smoker (*dark circles*). The neutral loss of 2′-deoxyribose from positively ionized 2′-deoxynucleoside putative adducts was analyzed by LC/ESI–MS/MS in MRM mode transmitting the $[M + H]^+ > [M + H - 116]^+$ transition over a total of 374 transitions in the mass range from *m/z* 228.8 to *m/z* 602.8. *Boxes A* through *C* define zones of activity that are discussed in the text. The *arrow* indicates the presence of a large putative adduct detected in the DNA of the smoker that was 4.8 times larger than in the DNA of the nonsmoker.

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Protein adductomics?

- Protein adducts have longer residence times than urinary adducts (28 d for HSA and 63 d for Hb)
- Proteins much more abundant than DNA in blood (1 ml contains 150 mg Hb, 30 mg of HSA, and 0.003-0.008 mg of DNA)
- HSA and/or Hb adducts have been related to some occupational or environmental exposures
  - Ethylene oxide, 1,3-butadiene, benzene, aflatoxin, PAHs, acrylamide

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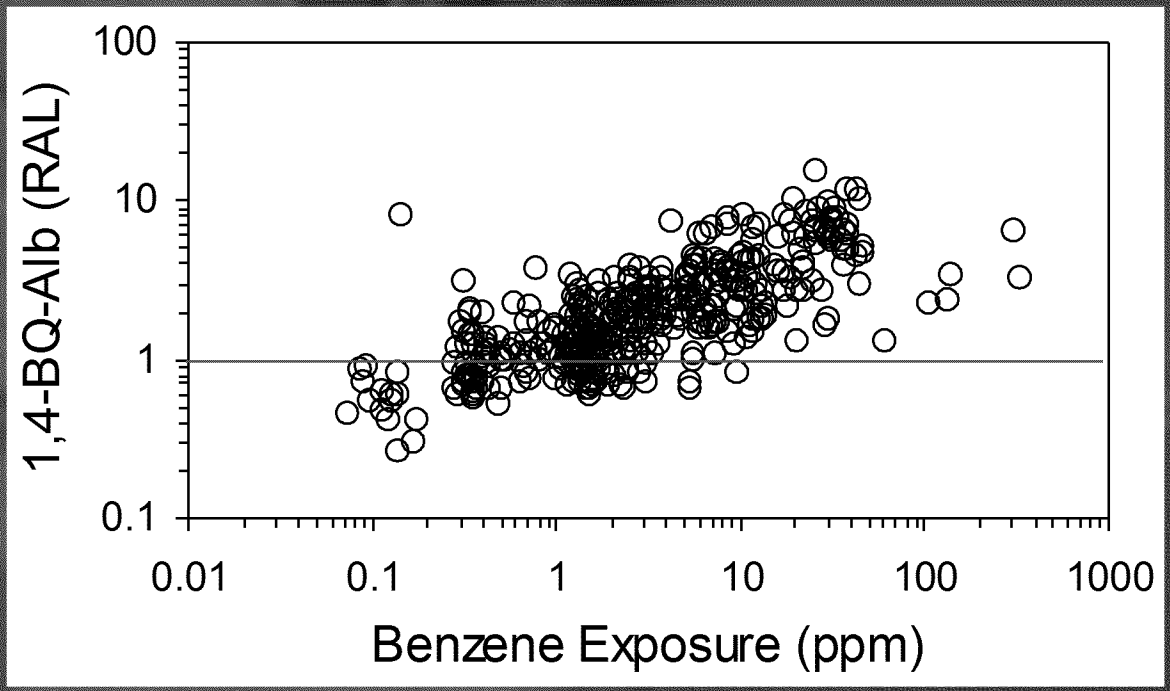
Benzoquinone adducts of HSA in benzene-exposed workers (*n* = 439)

Benzene → Phenol → Hydroquinone → Benzoquinone



Background adducts:

- Environmental benzene
- Hydroquinone in food and cigarette smoke
- Endogenous production of phenol

Data from: Yeowell O'Connell et al. *CEBP* (2001); Rappaport et al. *Cancer Res* (2002); Lin et al. *EHP* (2007)

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Human Serum Albumin (HSA)

- Most abundant protein in serum
- 585 Amino acids
- 35 Cys (-SH) residues
  - 34 Used for disulfide bonds
- One free thiol (Cys^{34})
  - Represents 90% of free thiols in serum
  - Scavenges ROX and other electrophiles
- *Preferred site for adduction of electrophiles in serum (conserved in all mammalian species)*

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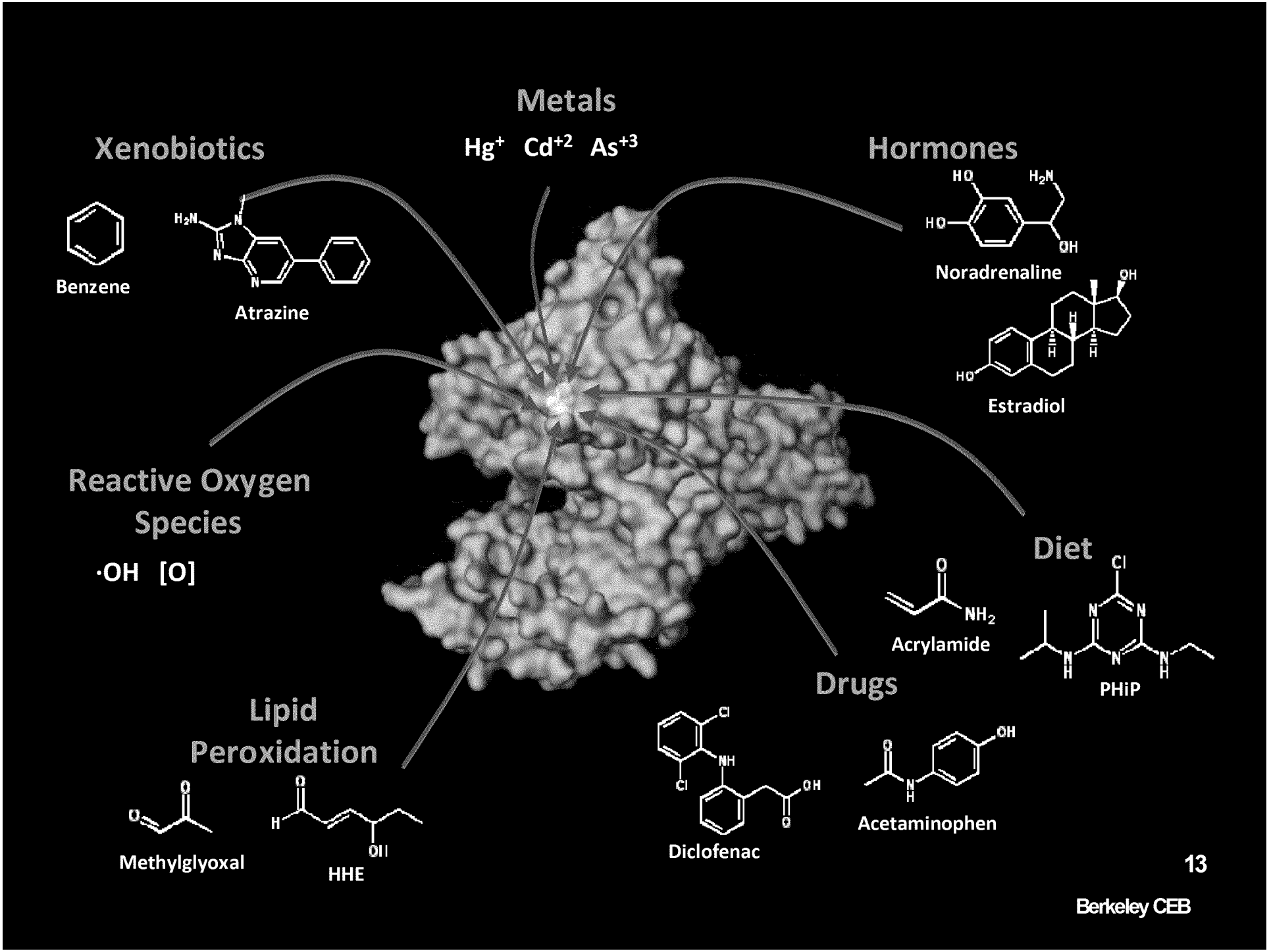
Metals
Hg^{+} Cd^{+2} As^{+3}
Xenobiotics
Benzene
Atrazine
Hormones
Noradrenaline
Estradiol
Reactive Oxygen Species
·OH [O]
Diet
Acrylamide
PHiP
Drugs
Diclofenac
Acetaminophen
Lipid Peroxidation
Methylglyoxal
HHE
13
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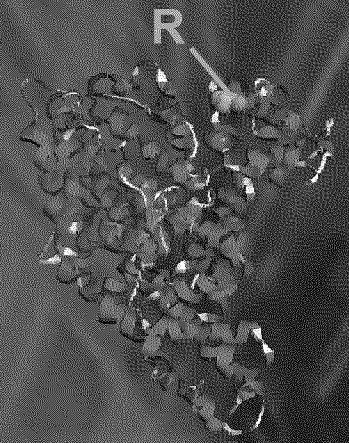
Scheme for characterizing adducts of HSA-Cys^{34}

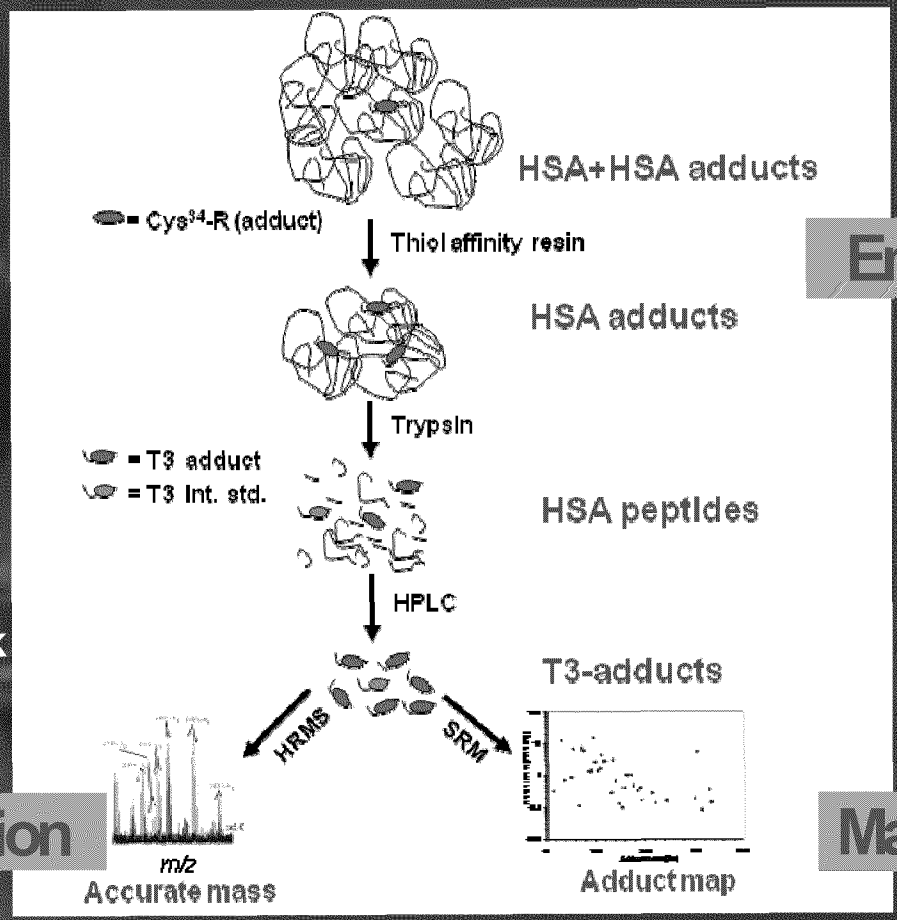


HSA Adduct

R

ALVLIAFAQYLQQCPVEDHVK

T3 Adduct



Enrichment

Identification

Mapping

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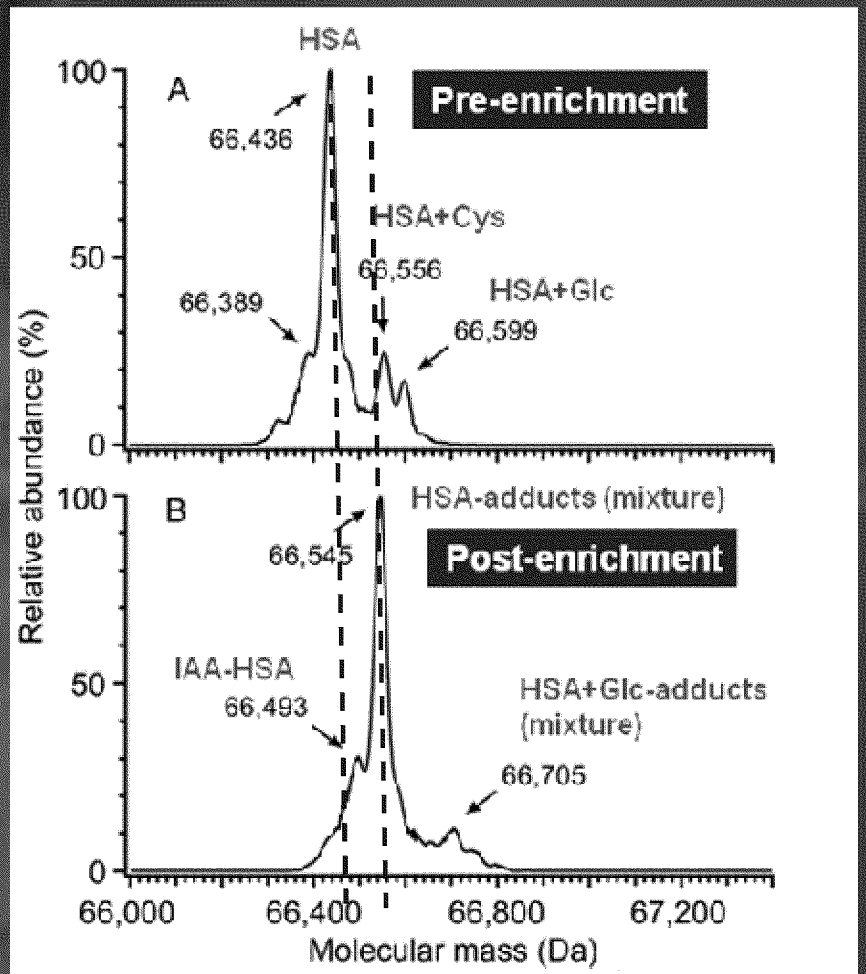
Adduct enrichment

HSA from a volunteer subject was analyzed by OrbiTrap MS (HSA-IAA was added as a positive control)

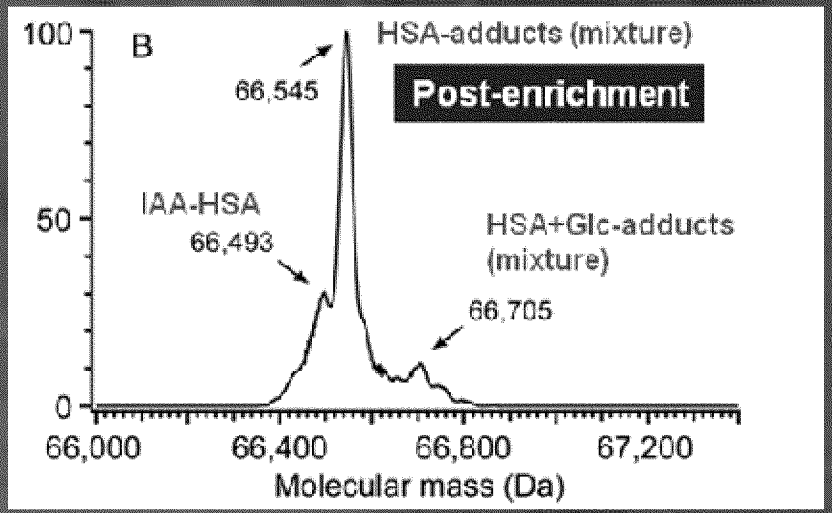


- Average adduct mass = 66,545-66,436 = 109 Da.
- About 3% of HSA is adducted

W. Funk *et al. Analyt Biochem,* in press.

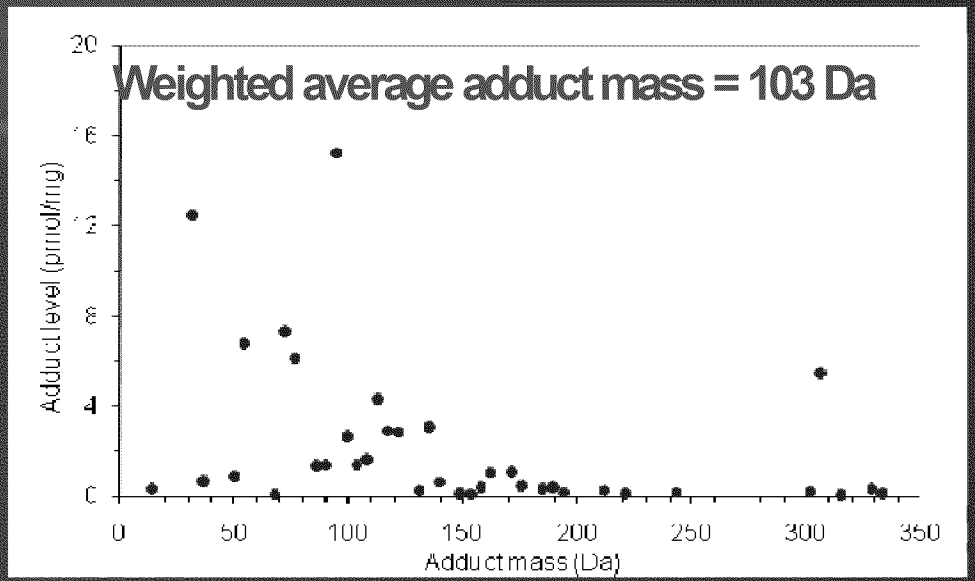
Adduct mapping

Average adduct mass = 109 Da



- 37 Points contained one or more adducts
- Mass range: 12 - 335 Da
- Abundance range: 0.08 – 15 pmol/mg HSA
- Avg. abundance = 2 pmol/mg (0.015% of HSA) suggesting 200 expected adducts

Tryptic digest of 0.5 mg HSA from the same subject (312 SRM-specific transitions for T3 adducts)



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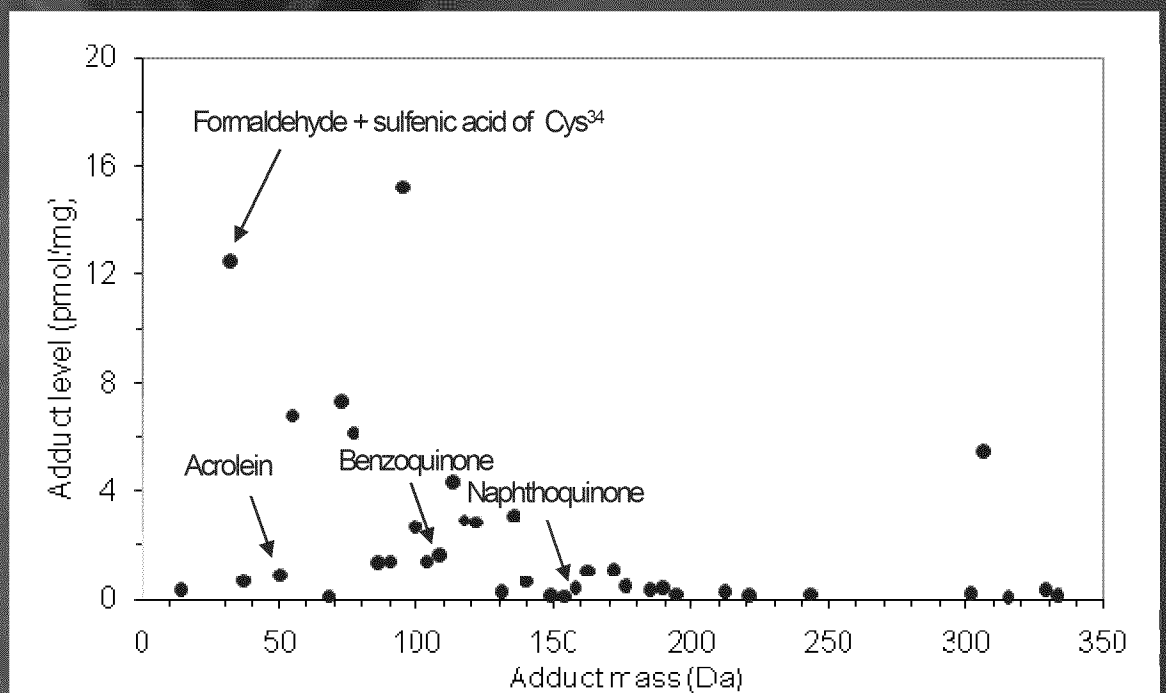
Adduct identification

Some adducts provided accurate masses via HRMS



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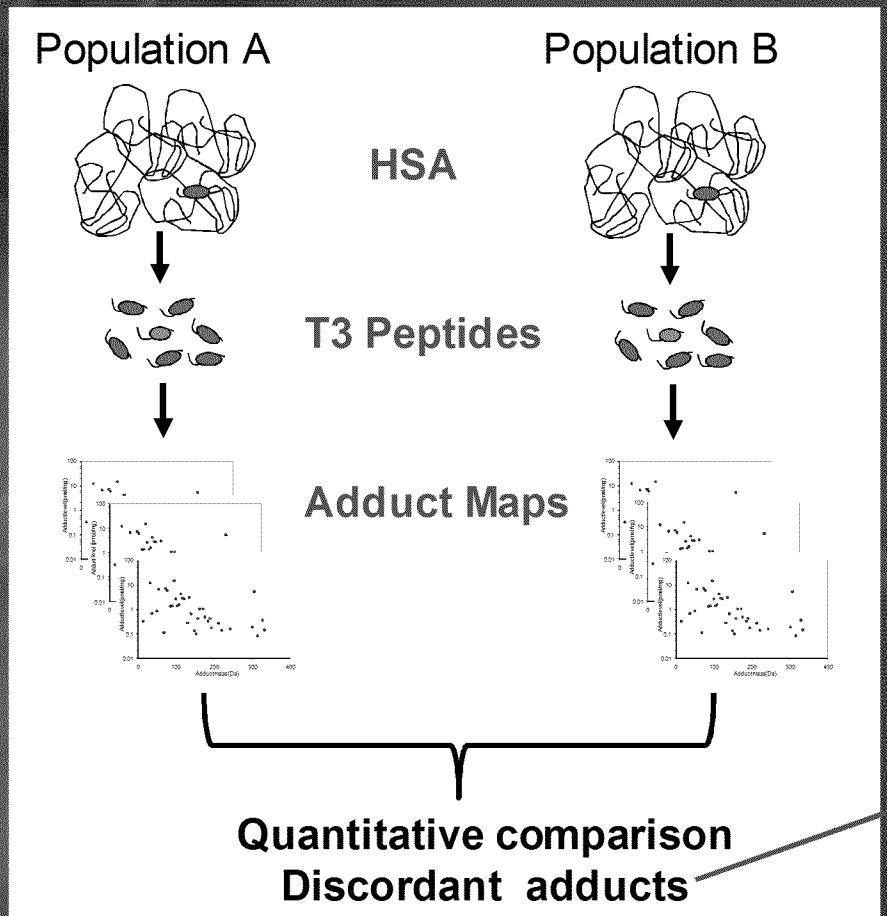
Adductomics and biomarker discovery



Currently comparing adduct maps between smokers & nonsmokers and between lymphoma cases & controls

Possible biomarkers of exposure or disease status

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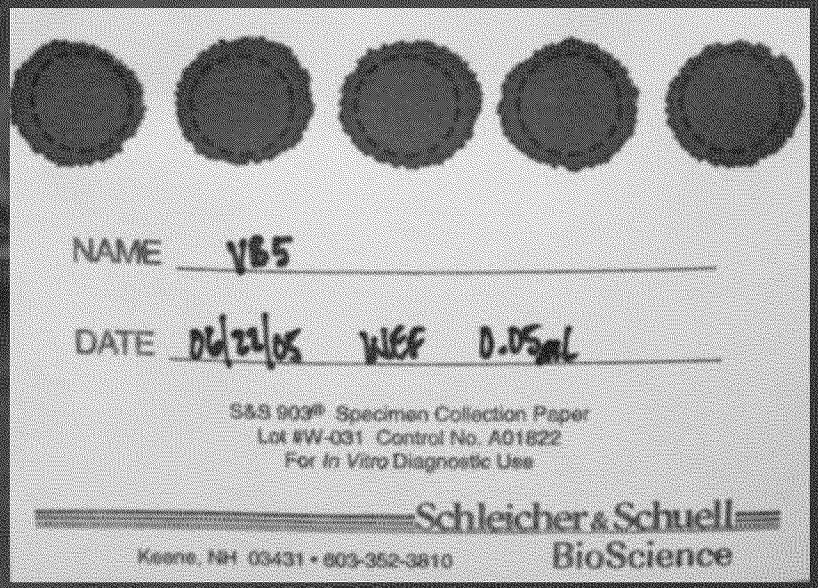
Adductomics with dried blood spots (DBS)

- One DBS contains 800 μg HSA (50 μl blood)
- SRM can detect HSA-Cys^{34} adducts in 1/8 – 1/4 of a DBS (3-mm or 6-mm punch)



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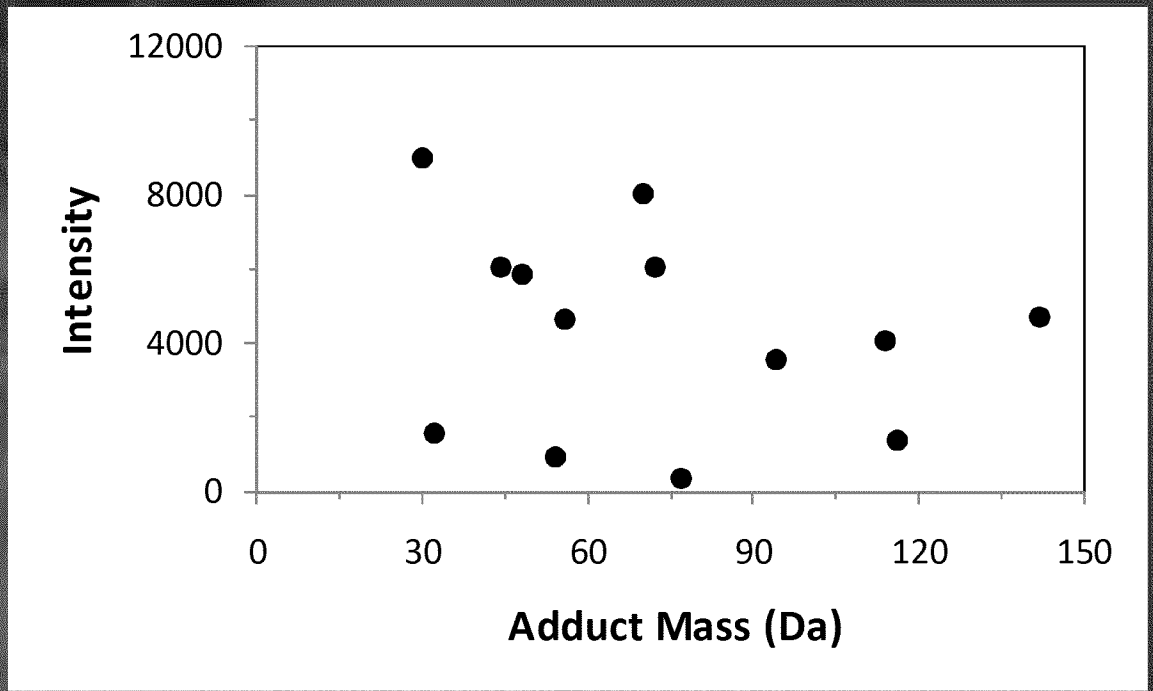
HSA-Cys^{34} adducts in DBS

Fresh DBS from a volunteer subject (6-mm punch)



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Fetal dried blood spots and childhood leukemia

- HSA adducts reflect exposures during the last month of gestation
- The Northern California Childhood Leukemia Study has DBS from 1000 cases and controls (P. Buffler *et al.*)
  - *HSA adducts will be measured in a subset of these DBS*

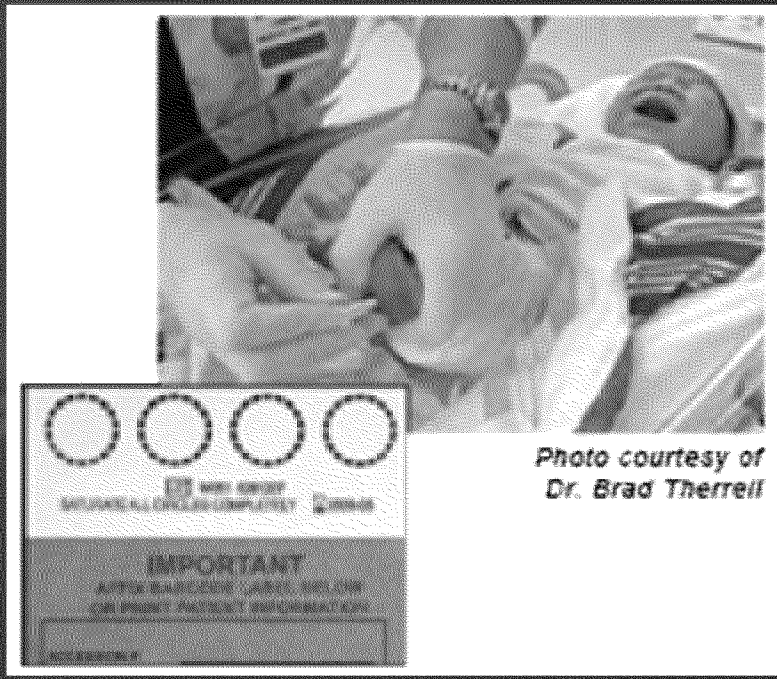

Photo courtesy of Dr. Brad Therrell

Measuring HSA adducts

Technology	Time frame	Volume of blood (ml)	Sensitivity (M)	Multiplex	Samples/d per analyst
Test tubes, handwritten labels, GC-MS	1980-1990	0.1 - 1	pM - nM	No	1 - 10
96-Well plates, bar codes, ELISA	1990-2000	0.01 – 0.1	fM - pM	No	10 - 100
Robotics, bar codes, SRM	2000-2010	0.01 – 0.1	aM - fM	Yes	10 - 100
Microfluidics, ELISA	2010 - 2020	0.001 – 0.01	aM - fM	??	100 - 1000

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Adductomics and P4 medicine

"P4 medicine is what I think is going to happen over the next 20 years. Medicine will move from its current largely reactive state to one that is *predictive*, that is *personalized*, that is eventually *preventive* and *participatory*… The idea would be that nanotech and microfluidic measurements will make it possible to have a device in the home that could prick the thumb, take a fraction of a droplet of blood, make 2500 measurements, … and send the information via wireless to a server."

Adductomics could provide some of these measurements!

A Conversation with Dr. Leroy Hood: Visionary Biologist and Biotechnologist
Paul S. Weiss, ACS Nano, 2007, 1 (4), pp 242–247.

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